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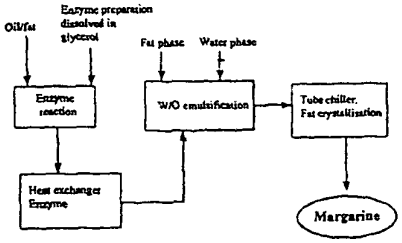
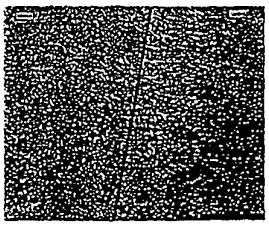
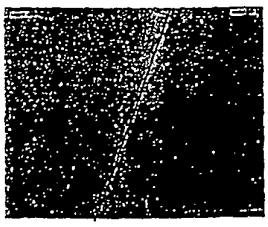
(57) Abstract

There is provided use of a conversion agent to prepare from a food material a foodstuff comprising at least one functional ingredient, wherein the at least one functional ingredient has been generated from at least one constituent of the food material by the conversion agent.







Foodstuff

The present invention relates to a foodstuff. More particularly, the present invention relates to a foodstuff comprising at least one functional ingredient which has been generated *in situ* by a conversion agent.

Traditionally food was prepared in the private households and the constituents of the food or of the foodstuff were brought to the kitchen of the household where the food or foodstuff was prepared shortly before consumption.

Industrial development increased the demand for the reduction of the time and effort required to prepare food or foodstuffs. Thus there has been a massive expansion in the industrial preparation of food.

Recently, there has been increased demand for improvements in the quality of industrially prepared food. In particular there is demand for improved taste, eating quality and shelf life. In an attempt to address these demands for improved foodstuffs, industrial food producers have utilised and have relied upon functional ingredients to meet the demands for quality and shelf life. Functional ingredients such as emulsifiers, hydrocolloids, preservatives, antioxidants, colourings and flavourings are widely used in the food industry.

More recently, there has been demand from consumers to reduce the number of additives, such as functional ingredients, included in foodstuffs. Thus, there is a desire to prepare industrially foodstuffs meeting the quality requirements of consumers whilst minimising the number of additives in the final foodstuffs.

Both Douglas B. Sarney *et al.*, Enzymatic Synthesis of Sorbitan Esters Using a Low-Boiling-Point Azeotrope as Reaction Solvent, Biotechnology and Bioengineering, 1997 vol. 54(4) and J. A. Arcosm *et al.*, Quantitative Enzymatic Production of 6.O-Acylglucose Esters, Biotechnology and Bioengineering 1998 57(5), teach the use lipase for the production of emulsifiers. The teachings require the synthesis of emulsifiers in

an organic solvent system. The emulsifier is then isolated from the organic solvent system before use in food.

A. Coteron *et al.*, Reactions of Olive Oil and Glycerol over Immobilised Lipases, JAOCS, Vol. 75, no. 5 (1998) reports the use of immobilised lipase in the reaction of olive oil and glycerol. Subsequent to the reaction the immobilised lipase is removed from the reaction mixture.

JP-A-90188214 reports the use of an immobilised lipase for the hydrolysis and ester exchange of triglyceride. In this process part of the triglyceride is partially hydrolysed to free fatty acid. The partially hydrolysed triglyceride product is used for production of margarine.

US-A-5,288,619 relates to enzymatic methods for the production of oils or fats having a specific fatty acid profile. In particular, US-A-5,288,619 discloses the use of a lipase to transesterification two oils or fats. A particularly preferred embodiment of this document uses an immobilised lipase. The resultant oils or fats, the required specific fatty acid profile, may subsequently be incorporated in a foodstuff or food material. For example the transesterified oils/fats may be incorporated in a margarine recipe.

US-A-4,865,866 teaches the use of a lipase to rearrange by transesterification the fatty acids components of a fat/oil. The disclosed lipases are immobilised, for example by support on Celite. The process is performed to provide a fat/oil composition having a specific fatty acid distribution. The fat/oil composition obtained by the transesterification may be incorporated in a foodstuff such as a plastic emulsion product e.g. a margarine or low fat spread.

JP-A-5211852 discloses the addition of a lipase to a mixture of water and less than 30% oil. The product prepared in this method may be used in the production of mayonnaise. The mayonnaise is prepared at a temperature such that the activity of lipase is not reduced. In the procedure of JP-A-5211852 the oil is degraded to free fatty acid or fatty acid salts(soap) and glycerol which may provide the emulsifying properties. However,

this may be problematic as the emulsification properties of this reaction product will depend on the pH of the mixture. This is because the effect of fatty acid is pH dependent. At low pH free fatty acid is present in the acid form which has low emulsification properties. At alkaline pH however free fatty acid is available as a soap, which is known to have good emulsification properties. For the production of a creamy substance described in JP-A-5211852 this may not be a problem. However, for other foodstuffs this may be a problem. For example, in margarine production pH is adjusted to 4.5 or 5.5 or other pH values depending on the recipe. In this case the effect of free fatty acid formation by the lipase will impact on the emulsification of the foodstuff.

The present invention addresses the problem of the prior art

According to a first aspect of the present invention there is provided use of a conversion agent to prepare from a food material a foodstuff comprising at least one functional ingredient, wherein the at least one functional ingredient has been generated from at least one constituent of the food material by the conversion agent.

According to a second aspect of the present invention there is provided a process for preparing a foodstuff comprising the steps of (i) providing a food material; (ii) contacting the food material with a conversion agent such that a functional ingredient is generated by the conversion agent from at least one constituent of the food material.

According to a third aspect of the present invention there is provided a foodstuff prepared from a food material, wherein the foodstuff comprises at least one functional ingredient, and wherein the at least one functional ingredient has been generated from at least one constituent of the food material by a conversion agent.

By the term "functional ingredient" we mean a constituent of the foodstuff which performs a specific function in the foodstuff. Preferably by the term "functional ingredient" we mean an emulsifier, hydrocolloid, preservative, antioxidant, colouring, flavouring, and/or viscosity modifier. Preferably by the term "functional ingredient" we mean a constituent of the foodstuff which has one or more of surface active properties,

antioxidative effect, anti-bacterial effect including bacteriostatic effect and/or bactericidal effect and viscosity modifying effect, preferably viscosity improving effect.

By the term "foodstuff" we mean a substance which is suitable for human or animal consumption.

The above aspects of the present invention are advantageous as they overcome the problems associated with the prior art.

The present invention utilises a conversion agent, such as enzyme, during the production of a foodstuff to generate one or more functional ingredients, for example emulsifiers, antioxidants or preservatives, from a constituent of a food material (i.e. ingredients) from which the foodstuff is prepared. The constituent(s) may be a fat, for example. Thus, instead of adding food additives produced by traditional chemical synthesis, the present invention provides for the *in situ* synthesis of a required functional ingredient.

Traditional chemical synthesis of functional ingredients is problematic because syntheses are often carried out under extreme conditions, such as high temperatures (e.g. ~200°C). Under extreme conditions, side reactions may occur. Thus, although the resultant product may be substantially pure, it may contain undesirable components. To eliminate undesirable components, reactions must be closely controlled and/or the resultant product may require purification, adding to a production process. The present invention aims to overcome these disadvantages.

Moreover, by generating the functional ingredient from at least one constituent of the food material using a conversion agent, the foodstuff comprises at least one less "additive" material. This is advantageous because of the improvement in the ease of production. Moreover, the foodstuff may contain less "additives". The reduction or elimination of "additives" is desirable to consumers and inclusion of additives often must be declared to the consumer in the ingredients listing on the foodstuff. Thus, the present invention is further advantageous.

As one of the advantages of the present invention is the possibility of providing a foodstuff prepared from a food material and comprising a functional ingredient which has been generated from a constituent of the food material, the following two aspects are preferred embodiments of the present invention

- in one preferred aspect the food material is substantially free of one of the at least one functional ingredients. In this aspect one of the functional ingredients must have been prepared at least in part in accordance with the present invention. By the term "substantially free" we mean the amount of the functional ingredient present in the food material is less than 10 % of the amount of the same functional ingredient present in the foodstuff, more preferably less than 5 %, more preferably less than 2 %, more preferably less than 1 %, yet more preferably less than 0.5 %
- in a further preferred aspect substantially all of at least one of the functional ingredients present in the foodstuff has provided by conversion in accordance with the present invention, together optionally with any of the functional ingredient present in the food material. By the term "substantially all" we mean the amount of the functional ingredient present in the foodstuff provided by conversion in accordance with the present invention, together optionally with any of the functional ingredient present in the food material, is greater than 90 % of the total amount of the functional ingredient, more preferably greater than 95 %, more preferably greater than 98 %, more preferably greater than 99 %.

The food material may be contacted with the conversion agent in any manner. The food material may be contacted with the conversion agent in an immobilised form. The food material may simply be added to the conversion agent or *vice versa*. In the latter aspect, the conversion agent may be subsequently removed from the food material/foodstuff or may remain in the food material/foodstuff. In a preferred aspect the conversion agent is present in the foodstuff.

The above preferred aspect is advantageous because one may contact the food material with the conversion agent to thereby provide a foodstuff suitable for consumption. No further processing or addition of ingredients may be required; a foodstuff comprising a

required functional ingredient is produced. Thus a foodstuff may be provided in which a required functional ingredient has been simply generated. Synthesis of the functional ingredient discretely from the foodstuff followed by subsequent addition is not required. Moreover, provided of course the conversion agent is suitably chosen so that it is compatible with a foodstuff i.e. it is edible, further processing of the foodstuff may not be necessary. However, the present invention encompasses foodstuffs which have been further processed.

Preferably, the conversion agent is a catalyst.

In a preferred aspect, the conversion agent is an enzyme. This aspect is particularly preferred because enzymes are readily available, may be chosen to convert a specific constituent of the food material and/or may be chosen to generate a specific functional ingredient. Yet further, enzymes may be denatured by heat. Thus in a further preferred aspect, the foodstuff/food material is heated after generation of the functional ingredient. The enzyme will be denatured and may then constitute protein. This is advantageous because the denatured enzyme need not be declared on the foodstuff/food material ingredients.

The use of enzymes is advantageous because denatured enzymes are considered, particularly under food labelling regulations, to constitute a processing aid. Inactivated enzymes are not considered to be additives; the addition of additives to foodstuffs is undesirable to many consumers.

Inactivation of the conversion agent, in particular denaturation of the enzyme, is advantageous because it allows one to control the amount of functional ingredient generated. For example, the generation of the functional ingredient may be monitored (for example by measurement of the functional properties of the food material) or the rate thereof determined. One may then terminate the generation of the functional ingredient, when a suitable amount of functional ingredient has been generated, by heating the food material. Thus the amount of the functional ingredient and the properties of the food material/foodstuff may easily be controlled.

Preferably, the enzyme is selected from lipases (EC 3.1.1.3), esterases, amylases, xylanases, proteases, lyases, including glucan lyases and α-1,4-glucan lyase, derivatives and mixtures thereof. More preferably, the enzyme is selected from lipases, esterases, derivatives and mixtures thereof.

Preferably the enzyme is an enzyme as described in and/or as claimed in Danish Patent Application No. 0400/97. In other words preferably the enzyme is a polypeptide in glycosylated or non-glycosylated form capable of exhibiting lipase activity wherein the polypeptide comprises at least one amino acid sequence selected from the group consisting of

(I) Ser-Val-Ser-Thr-Ser-Thr-Leu-Asp-Glu-Leu-Gln-Leu-Phe-Ala-Gln-Trp-Ser-Ala-Ala-Ala-Tyr-Xaa-Ser-Asn-Asn

(II) Val-His-Thr-Gly-Phe-Trp-Lys

(III) Ala-Trp-Glu-Ser-Ala-Ala-Asp-Glu-Leu-Thr-Ser-Lys-Ile-Lys

where Xaa represents an amino acid selected from the group consisting of Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr and Val.

In a further aspect the enzyme may be an enzyme as described in and/or as claimed in International Patent Application No. PCT/IB98/00708, filed 6 May 1998.

Preferably, the enzyme is isolated from a plant (preferably soy bean, rice bran, corn, rapeseed, peanut, pineapple, potato, oat, wheat and/or sunflower seed), an animal (preferably an animal pancreas) or a micro-organism. Preferably, the micro-organism selected from *Aspergillus niger, Rhizopus delemar, Rhizopus arrhizus, Mucor miehei, Pseudomonas sp., Candida rugosa, Pencilium roqueforti, Pencilium cyclopium, Aspergillus tubingensis, Candida cylindracea, Thermomyces lanuginosus, Mucor*

javanicus, Candida antarctica, Chromobacterium viscosum, Pseudomanas fluorescens, Pseudomonas nitroreducans, Chromobacterium viscosum, Bacillus subtilis, mutants and combinations thereof.

Preferably, the conversion agent is present in the foodstuff. More preferably, the conversion agent is present in an inactive form or in a denatured form in the foodstuff.

In one aspect of the present invention the at least one functional ingredient may be generated from the at least one constituent of the food material by two or more conversion agents. The at least one constituent may be contacted with the two or more conversion agents at the same time or in series or a combination thereof.

Preferably, the at least one constituent of the food material is selected from esters, monoglycerides, diglycerides, triglycerides, fats, including lard, tallow and butter fat; fatty acids, fatty acid esters, waxes, wax esters, oils including oils extracted from or derived from palm oil, sunflower oil, soya bean oil, safflower oil, cotton seed oil, ground nut oil, corn oil, olive oil, peanut oil, coconut oil and rape seed oil, proteins, amino acids, protein hydrolysates, peptides (partly hydrolysed protein), a constituent comprising a hydroxy group (-OH), polyvalent alcohols, including glycerol; water, ethanol, sugars including sucrose, fructose, glucose (dextrose), lactose, and galactose; dextrins including maltodextrin, sorbitol, mannitol, fruit acids and hydroxy acids including citric acid, tartaric acid, lactic acid and ascorbic acid; proteins, amino acids, protein hydrolysates, peptides (partly hydrolysed protein); mixtures and derivatives thereof.

Preferably, the at least one constituent of the food material is in liquid form.

The term "triglyceride" preferably means a triester of an alcohol, preferably glycerol, and a fatty acid. More preferably the triglyceride fatty acid is a triester of an alcohol, preferably glycerol, and a C4 to C24 fatty acid. Preferably the triglyceride fatty acid has an iodine value of from 0 to 125, preferably from 0 to 60.

Preferably, the triglyceride is selected from triglycerides having a fatty acid chain length of no greater than 14 carbons, triglycerides having a fatty acid chain length of from 4 to 14 carbons, triglycerides having a fatty acid chain length of from 6 to 14 carbons, triglycerides having a fatty acid chain length of from 8 to 14 carbons, triglycerides having a fatty acid chain length of from 10 to 14 carbons, triglycerides having a fatty acid chain length of 12 carbons, triglycerides having a fatty acid chain length of from 16 to 24 carbons, triglycerides having a fatty acid chain length of from 16 to 22 carbons, triglycerides having a fatty acid chain length of from 18 to 22 carbons, triglycerides having a fatty acid chain length of from 18 to 20 carbons, mixtures and derivatives thereof.

Preferably, the functional ingredient is generated from at least two constituents of the food material. In this aspect at least two constituents of the foodstuff may interact and/or react and/or combine together to generate at least one functional ingredient. Preferably, the functional ingredient is generated from a first constituent and a second constituent of the food material.

Preferably, the first constituent and the second constituent are constituents of the foodstuff. In this aspect, the functional ingredient is generated from a first constituent and a second constituent of the food material and the first constituent and second constituent are also present in the foodstuff. Thus the functional ingredient may be generated from constituents/ingredients of the food material which are only partially used to generate the functional ingredient. The remainder of the constituents/ingredients may be present in the foodstuff.

In a preferred aspect of the present invention the first constituent of the food material/foodstuff is hydrophobic and/or lipophilic.

Preferably, the first constituent of the food material/foodstuff is selected from esters, monoglycerides, diglycerides, triglycerides, fats, including lard, tallow and butter fat; fatty acids, fatty acid esters, waxes, wax esters, oils including oils extracted from or derived from palm oil, sunflower oil, soya bean oil, safflower oil, cotton seed oil, ground nut oil, corn oil, olive oil, peanut oil, coconut oil and rape seed oil; derivatives

and mixtures thereof. More preferably, the first constituent of the food material/foodstuff comprises or is an ester or a triglyceride.

The term "triglyceride" preferably has the meaning defined above.

Preferably, the triglyceride of the first constituent is selected from triglycerides having a fatty acid chain length of no greater than 14 carbons, triglycerides having a fatty acid chain length of from 4 to 14 carbons, triglycerides having a fatty acid chain length of from 6 to 14 carbons, triglycerides having a fatty acid chain length of from 8 to 14 carbons, triglycerides having a fatty acid chain length of from 10 to 14 carbons, triglycerides having a fatty acid chain length of 12 carbons, triglycerides having a fatty acid chain length of from 16 to 24 carbons, triglycerides having a fatty acid chain length of from 16 to 22 carbons, triglycerides having a fatty acid chain length of from 18 to 22 carbons, triglycerides having a fatty acid chain length of from 18 to 20 carbons, mixtures and derivatives thereof.

Preferably, the first constituent of the food material/foodstuff is in liquid form.

In a preferred aspect of the present invention the second constituent of the food material/foodstuff is hydrophilic.

In a preferred embodiment, the second constituent of the food material/foodstuff may be selected from proteins, amino acids, protein hydrolysates, peptides (partly hydrolysed protein), mixtures and derivatives thereof.

In this aspect, wherein the first constituent of the food material/foodstuff is preferably a fatty acid, it is possible to esterify the free amino groups in the proteinatious second constituent with fatty acid from the first constituent. In this manner, it is possible to produce protein fatty acid condensate. Alternatively, the present invention provides a process; in which the first constituent of the food material/foodstuff is selected from esters, monoglycerides, diglycerides, triglycerides, fats (including tallow and lard), fatty acid esters, and oils (including palm oil, and soya oil rape seed oil), and in which the

second constituent is proteinatious; wherein the first constituent interesterifies with the proteinatious second constituent. In these manners, it is possible to produce protein fatty acid condensate.

Protein fatty acid condensate has very good surface active properties. Protein fatty acid condensate is known within the cosmetic and textile industry (see Herstellung und Anvendungmöglichkeiten von Eiweiss-Fettsäurekondensaten. Andreas Sander, Eberhard Eilers, Andrea Heilmann, Edith von Kreis. Fett/lipid 99 (1997) Nr. 4, 115-120). This condensate is normally produced by a reaction between protein and fatty acid chloride as disclosed in Sander et al. However, enzymatic processes for the production of protein fatty acid condensate from protein and fatty acid is known (WO 97/14713). The present applicants have identified that by utilising the commonly occurring constituents of food material, an emulsifier in the form of protein fatty acid condensate may be provided.

This is particularly advantageous because protein forms part of many types of food and is the basic material in many products, for example meat products. In the food industry protein is also often used as a purified protein isolated from milk and plants, such as soya, wheat, rice. Protein is also prepared and is available in hydrolysed form, i.e. protein hydrolysate, peptides or amino acids.

In the above aspect of the present invention, wherein a protein fatty acid condensate is formed, it is important to contact the first constituent and the second constituent with the conversion agent under conditions of agitation. Moreover, it is important to contact these constituents under conditions of controlled water activity. Both of these preferred features will assist in obtaining a maximum conversion rate of first constituents/second constituent to functional ingredient.

Preferably, the second constituent of the food material/foodstuff is selected from a constituent comprising a hydroxy group (-OH), polyvalent alcohols, including glycerol; water, ethanol, sugars including sucrose, fructose, glucose (dextrose), lactose, and galactose; dextrins including maltodextrin, sorbitol, mannitol, fruit acids and hydroxy

acids including citric acid, tartaric acid, lactic acid and ascorbic acid; mixtures and derivatives thereof. More preferably, the second constituent of the food material/foodstuff is glycerol.

In a further preferred embodiment, the first constituent of the food material/foodstuff is an ester, preferably a triglyceride and the second constituent of the food material/foodstuff is a constituent comprising a hydroxy group (-OH). Preferably, the first constituent of the food material/foodstuff is a triglyceride. Preferably, the second constituent of the food material/foodstuff is an alcohol, more preferably a polyvalent alcohol, yet more preferably glycerol.

Preferably, the second constituent of the food material/foodstuff is in liquid form.

In a highly preferred embodiment, the first constituent of the food material/foodstuff is a constituent comprising at least two ester groups, preferably a triester, more preferably a triglyceride and the second constituent of the food material/foodstuff is a sugar or a sugar alcohol. In this highly preferred aspect the first constituent and the second constituent may interact on contact with the a conversion agent to generate an ester derived from the first constituent wherein the ester has a lower degree of esterification than the first constituent, and a sugar ester. This is extremely advantageous because the ester may act as a functional ingredient, such as an emulsifier, and the sugar ester may also act as a functional ingredient, such as an emulsifier or an anti-oxidant. Thus, two functional ingredients may be generated from two constituents of the food material/foodstuff by a conversion agent.

In the above highly preferred aspect the second constituent is preferably ascorbic acid. Ascorbic acid ester is an antioxidant.

Thus, in a further broad aspect of the present invention there is provided a foodstuff prepared from a food material, wherein the foodstuff comprises at least two functional ingredients, and wherein the at least two functional ingredients have been generated from a first constituent of the food material and a second constituent of the food

material by a conversion agent. Preferably, the first constituent is a constituent comprising at least two ester groups, preferably a triester, more preferably a triglyceride. Preferably, the second constituent is a sugar or a sugar alcohol, more preferably ascorbic acid.

In a preferred aspect, the first constituent of the food material/foodstuff and the first constituent of the food material/foodstuff are in liquid form.

In a further preferred aspect, the food material/foodstuff further comprises greater than two constituents. Preferably, the food material/foodstuff further comprises a third constituent. The third constituent may be selected from the constituents listed above in respect of the first and second constituents. Preferably, the third constituent is selected from a constituent comprising a hydroxy group (-OH), polyvalent alcohols, including glycerol; water, ethanol, sugars including sucrose, fructose, glucose (dextrose), lactose, and galactose; dextrins including maltodextrin, sorbitol, mannitol, fruit acids and hydroxy acids including citric acid, tartaric acid, lactic acid and ascorbic acid; mixtures and derivatives thereof.

Preferably, the third constituent of the food material/foodstuff is selected from sugars including sucrose, fructose, glucose (dextrose), lactose, and galactose; dextrins including maltodextrin, sorbitol, mannitol, fruit acids and hydroxy acids including citric acid, tartaric acid, lactic acid and ascorbic acid; mixtures and derivatives thereof.

In a highly preferred aspect of the present invention the second constituent of the food material/foodstuff is selected from polyvalent alcohols, preferably glycerol, and the third constituent of the food material/foodstuff is selected from sugars. In an alternative highly preferred aspect of the present invention the second constituent of the food material/foodstuff is selected from polyvalent alcohols, preferably glycerol, and the third constituent of the food material/foodstuff is selected from proteins, peptides and amino acids. These preferred aspects are advantageous because the third constituent may be soluble in the second constituent. Thus, the second constituent can readily react with the third constituent. Moreover, when the first constituent of the food

material/foodstuff is in liquid form, the second constituent and/or the third constituent can readily react with the first constituent.

The provision of one or more constituents in liquid form as described above may significantly increase the reaction velocity of the generation of the least one functional ingredient.

The conversion agent may be contacted with the all of the food material or a portion thereof. In the former case, a portion of the food material is contacted with the conversion agent and the contacted material is subsequently contacted with the further constituents of the food material. In the latter case, a portion of the food material may be removed from the total amount of food material. After contacting the conversion agent with the portion of food material, the portion may be returned to the remainder of food material. The portion of the food material may comprises from 0.1 to 10 wt % of the total food material, preferably from 0.1 to 5 wt % of the total food material, preferably from 0.1 to 2 wt % of the total food material, more preferably from 0.5 to 1 wt % of the total food material.

An Example of a portion of the food material being contacted with the conversion agent and the contacted material subsequently being contacted with the further constituents of the food material is exemplified in Figure 1 (Flow diagram for *in situ* production of emulsifier). Figure 1 illustrated the contact of an enzyme with an oil/fat to provide a composition comprising an emulsifier. The enzyme present in the contacted food material is then inactivated with heat. The emulsifier containing foodstuff is then mixed with a fat phase and a water phase and fed to tube chiller to provide a water-in-oil margarine.

The conversion agent may be contacted with a carrier prior to contact with the food material. Preferably, the carrier is a constituent of the food material. Preferably, the carrier is a first constituent or a second constituent of the food material as defined above. More preferably, the carrier is glycerol.

The conversion agent may be contacted with the food material under supercritical

conditions. In this aspect the conversion agent may be contacted with the food material in a carbon dioxide solvent. Preferably, the carbon dioxide solvent comprises a mixture of carbon dioxide and an alcohol.

Preferably, the functional ingredient of the present invention is generated by a reaction selected from alcoholysis, preferably glycerolysis, hydrolysis, interesterification, and combinations thereof. More preferably the functional ingredient is generated by a alcoholysis reaction, preferably a glycerolysis reaction.

Preferably, the functional ingredient comprises less than 5 wt % of the foodstuff. Preferably, the functional ingredient comprises from 0.01 to 4 wt % of the foodstuff. Preferably, the functional ingredient comprises from 0.01 to 2 wt % of the foodstuff. Preferably, the functional ingredient comprises from 0.01 to 1 wt % of the foodstuff. Preferably, the functional ingredient comprises from 0.01 to 0.5 wt % of the foodstuff. Preferably, the functional ingredient comprises from 0.01 to 0.3 wt % of the foodstuff.

Preferably, the at least one functional ingredient comprises or is a functional ingredient selected from emulsifiers, hydrocolloids, preservatives, antioxidants, colourings and flavourings. More preferably, the at least one functional ingredient comprises or is an emulsifier. In this aspect, preferably the emulsifier comprises from 0.1 to 0.3 wt % of the foodstuff.

The emulsifier may comprise or may be selected from monoglycerides, diglycerides, derivatives and mixtures thereof.

The antioxidant may be anhydrofructose. In this aspect, the at least one constituent is preferably a glucan, more preferably a starch. In this aspect, the conversion agent is preferably a lyase enzyme, yet more preferably an enzyme as described in and/or as claimed in International Patent Application No. PCT/IB98/00708, filed 6 May 1998.

In one aspect of the present invention the at least one functional ingredient is other than an antioxidant. In a further aspect of the present invention the foodstuff does not

contain an antioxidant generated in accordance with the present invention. In a further aspect of the present invention the foodstuff does not contain an antioxidant.

In a further aspect of the present invention the food material and/or the food material contacted with the conversion agent and/or the conversion material is substantially free of water. In this aspect, the creation of free fatty acids and there presence in the foodstuff may be reduced or avoided when the food material is contacted with the conversion agent.

An example of the is aspect of the invention is provided wherein a lipase carried in glycerol, preferably in a glycerol/sugar mixture is contacted with a triglyceride. In this aspect advantageous mono-diglycerides and, preferable, sugar esters are generated as functional ingredients.

Triglyceride + glycerol	lipase →	mono- diglyceride and triglyceride
or:		
Triglyceride + glycerol/sugar	lipase →	mono- diglyceride and triglyceride + sugar esters

A person skilled in the art will appreciate that the at least one constituent of the food material from which the functional ingredient is generated may be selected to provide a required functional ingredient. Thus in the above aspect wherein the functional ingredient is an emulsifier, preferably an emulsifier selected from monoglycerides, diglycerides, derivatives and mixtures thereof, the at least one constituent may be, for example, a triglyceride and a polyvalent alcohol.

In a preferred aspect the present invention provides foodstuff as defined above wherein the foodstuff is selected from baked goods, including breads, cakes, sweet dough products, laminated doughs, liquid batters, muffins, doughnuts, biscuits, crackers and cookies; confectionery, including chocolate, candies, caramels, halawa, gums, including sugar free and sugar sweetened gums, bubble gum, soft bubble gum, chewing gum and puddings; frozen products including sorbets, preferably frozen dairy products, including

ice cream and ice milk; dairy products, including coffee cream, whipped cream, custard cream, milk drinks and yoghurts; mousses, whipped vegetable creams, meat products, including processed meat products; edible oils and fats, aerated and non-aerated whipped products, oil-in-water emulsions, water-in-oil emulsions, margarine, shortening and spreads including low fat and very low fat spreads; dressings, mayonnaise, dips, cream based sauces, cream based soups, beverages, spice emulsions, sauces and mayonnaise.

In one aspect of the present invention the foodstuff is a foodstuff other than mayonnaise.

The claims of the present application are to be construed to include each of the foodstuffs listed above.

In a preferred embodiment the foodstuff of the present invention is a spread, preferably a margarine.

Thus in a preferred aspect the present invention provides a margarine prepared from a food material, wherein the foodstuff comprises at least one functional ingredient, and wherein the at least one functional ingredient has been generated from at least one constituent of the food material by a conversion agent.

In a further preferred embodiment the foodstuff comprises greater than 30 wt % fat (i.e. triglycerides), more preferably greater than 40 wt % fat, yet more preferably greater than 50 wt % fat.

The foodstuff may comprise an emulsion of oil and water. The emulsion may be an oil-in-water emulsion. The emulsion may be an water-in-oil emulsion.

The invention will now be described, by way of example only, with reference to the following examples.

EXAMPLES

Example 1 - Full-fat Table Margarine

Full-fat table margarine is used for spreading on bread and household baking.

Each of fat blends A, B and C listed in Table 1 were treated with lipase as follows. 1 part of the fat blend is heated to 50°C during stirring 0.2 part of lipase (obtained from *Aspergillus tubingensis)* dispersed in glycerol is added. The fat blend is reacted for 12 hours at 50 °C and then shortly heated to 100 °C to denature the enzyme.

Table 1

Fat blends	A	B	C
Margarine used at approx.	5-10°C	20-25°C	25-30°C
Soya 41°C	20	-	-
Soya 35°C	20	-	-
Soya oil	60	25	20
Palm 43°C	-	25	30
Palm oil	-	50	50
SFC* values of fat blends (IUPAC method)	A	B	C
5°C	34	47	54
10°C	28	45	50
20°C	14	26	30
30°C	3	10	12
35°C	0	5	7
40°C	0	1	2
Slip melting point °C (AOCS 3-25 method)	26.3	36.9	36.8

* Solid fat content

The treated fat blends were then processed in accordance with the following steps to prepare a recipe shown in Table 2

1. Blend the water phase ingredients. (If required, pasteurise the water phase by heating to approx. 80°C). Adjust pH with Ferment Flavouring 4646.
2. Melt the fat phase, and temper to approx. 40-45°C. Add the β-carotene.

3. Add the flavouring.
4. Add the water phase to the fat phase, stirring continuously.
5. Cool in a tube chiller (normal capacity, normal cooling) to an outlet temp of 8-10°C.

Table 2

Water phase:	Water	16.0%
	Skimmed milk powder or whey powder	1.0%
	Salt	1.0%
	pH 5.0-5.5 with Ferment Flavouring 4646*	
Fat phase:	Lipase treated fat	1.2%
	Soya lecithin	0.2%
	β-carotene	4 ppm
	Fat blend	80.6%
	Butter Flavouring 3559**	0.01%

* Ferment Flavouring 4646 - a nature-identical, water-soluble flavouring which ensures a good, lactic and fermented taste. Used for direct acidification of the water phase to ensure the taste similar to that obtained by microbial fermentation of the milk

** Butter Flavouring 3559 - a nature-identical, fat-soluble flavouring which provides a rich, fermented butter taste.

The fat contacted with lipase generated an emulsifier, a functional ingredient which is important in the preparation of margarine. Each of the margarines prepared from fat blends A, B and C was visually inspected and found to substantially identical in appearance to conventionally prepared margarine. No separation of the oil and water phases was observed. Each of the margarines prepared from fat blends A, B and C was also spread on bread and tasted. The organoleptic properties of each margarine was pleasant and were felt by the taster to be substantially identical to those of conventionally prepared margarine.

Example 2 - 60% Fat Spread with Protein

60% fat spread with protein is used for spreading on bread and open pan frying instead of full-fat products.

Each of fat blends A and B listed in Table 3 were treated with lipase as follows. 1 part of the fat blend is heated to 50°C during stirring 0.2 part of lipase (obtained from *Rhizopus arrhizus)* dispersed in glycerol is added. The fat blend is reacted for 12 hours at 50 °C and then shortly heated to 100 °C to denature the enzyme.

Table 3

Fat blends	A	B
Spread used at approx.	5-10°C	20-25°C
Soya 41°C	25	20
Soya 35°C	-	45
Soya oil	75	35
SFC values of fat blends (IUPAC method)	A	B
5°C	23	48
10°C	19	46
20°C	9	28
30°C	2	8
35°C	0	2
40°C	0	0
Slip melting point °C (AOCS 3-25 method)	26.6	31.7

The treated fat blends were then processed in accordance with the following steps to prepare a recipe shown in Table 4

1. Blend the water phase ingredients. (If required, pasteurise the water phase by heating to approx. 80°C). Adjust pH.
2. Melt the fat phase, and temper to approx. 40-45°C. Add the β-carotene.
3. Add the flavouring.
4. Add the water phase to the fat phase, stirring vigorously.
5. Crystallise and knead vigorously in a tube chiller (80% of normal capacity, NH_3 - 15°C, 2 tubes) to an outlet temperature of 8-10°C

Table 4

Water phase at pH 5.5:	Water	37.9%
	Whey powder	1.0%
	Salt	1.0%
	K-sorbate	0.1%
Fat phase:	Lipase treated fat	1.4%

	β-carotene	4 ppm
	Fat blend	58.6%
	Butter Flavouring 3559	0.01%

The fat contacted with lipase generated an emulsifier. Both of the margarines prepared from fat blends A and B was visually inspected and found to substantially identical in appearance to conventionally prepared margarine. No separation of the oil and water phases was observed. Both of the margarines prepared from fat blends A and B was also spread on bread and tasted. The organoleptic properties of each margarine was pleasant and were felt by the taster to be substantially identical to those of conventionally prepared margarine.

Example 3 - 40% Fat Spread with Whey Powder

Fat blend A listed in Table 3 above was treated with lipase as follows. 1 part of the fat blend is heated to 50°C during stirring 0.2 part of lipase (obtained from *Candida rugosa)* dispersed in glycerol is added. The fat blend is reacted for 12 hours at 50 °C and then shortly heated to 100 °C to denature the enzyme and used for 40% fat spread production. The spread had a composition shown in Table 5 below.

Table 5

Water phase at pH 5.5	Water	55.16%
	Salt	1.2%
	K-sorbate	0.1%
	Whey powder	1.0%
	GRINDSTED™ Pectin LFS 100	1.0%
Fat phase	Fat blend - 25 parts soya 41° - 75 parts liquid oil	39.5%
	β-carotene	4ppm
	Butter Flavouring 2873	0.01%
	Butter Flavouring 3507	0.01%
	Lipase treated fat	2.0%

The fat contacted with lipase generated an emulsifier. The low-fat spread was stable and had good water dispersion. Sensory evaluation of the sample showed that they had a very good flavour release and colour.

Example 4 - Filling Cream.

Each of fat blends A and B listed in Table 3 above were treated with lipase as follows. 1 part of the fat blend is heated to 45°C during stirring 0.2 part of lipase (obtained from *Rhizopus delemar)* dispersed in glycerol is added. The fat blend is reacted for 12 hours at 45 °C and then shortly heated to 100 °C to denature the enzyme and used for filling cream production..

Filling cream was made in a ice cream freezer with mono-pump (capacity 27 kg/hr). Nitrogen blown in after the pump and before the cooling cylinder. Outlet temperature: 15-17 °C.

The filling cream spread had a composition shown in Table 6 below.

Table 6

Water phase	Water	12.5%
	GRINDSTED™ Pectin LFS 100	0.5%
	SMP	8.0%
	Sucrose	9.9%
	Invert sugar	9.0%
	Sorbitol 70%	8.0%
	Glucose syrup	14.0%
	Glycerol	7.0%
	K-sorbate	0.1%
Fat phase	Lipase treated fat	3,0%
	Lecithin	0,4%
	Fat blend (100% coconut 31 °C)	27.6%
	Butter flavouring 2598	0.03%

The fat contacted with lipase generated an emulsifier. The filling cream was smooth with good flavour release. Specific gravity of the cream: 0.8 g/ml.

Example 5 - Ice cream

1 part of soya fat 41 ° is heated to 45 °C during stirring 0.2 part of lipase (obtained from *Aspergillus niger)* dispersed in glycerol is added. The fat blend is reacted for 12 hours at 45 °C and then shortly heated to 100 °C to denature the enzyme and used for ice cream production.

The treated fat was then processed in accordance with the following steps to prepare a recipes shown in Table 7

1. Heat all liquid ingredients to approx. 40 °C
2. Add dry ingredients. (stabiliser blend is mixed with sugar before addition)
3. If butter/butter oil or veg. fat is used it must be melted separately and added to the mix at 40 °C, or via a static mixer at the entrance to the homogeniser by means of a dosing pump.
4. Pasteurise at 80 -85 °C/20-40 seconds
5. Homogenise at 80 °C (190 bar for recipe 1 and 175 bar for recipe 2)
6. Cool to ageing temperature , 4 °C
7. Freeze in continuos freezer to desired overrun (100% recommended)
8. Harden in tunnel at -40 °C
9. Store below -25 °C

Table 7

Recipe	1 Milk fat	2 Veg. fat
Dairy cream, 38%	23.65	
Skimmed milk	53.30	
Skimmed milk powder	4.90	11.30
Vegetable fat (HCO)		8.00
Sugar	12.00	12.00
Glucose syrup, DE 42, 75% TS	5.25	5.25
Stabiliser blend	0.2	0.2
Lipase treated fat	0.6	0.6

Grindsted Flavouring 2976	0.1	0.1
Colour	+	+
Water		62.55

The fat contacted with lipase generated an emulsifier. Ice cream of both recipes had a good taste and excellent creamy mouthfeel.

Example 6 -Margarine

In a vessel 0.6 part of sun flower oil and 0.4 part of palm oil and 0.15 part of lipase from *Rhizopus arrhizus* dissolved glycerol/water is added. The reaction is continued for 20 hours at 45°C and then shortly treated by 100°C in order to inactivate the enzyme.

Two recipes were prepared. These recipes are shown in Table 8 below. Recipe 1 was in accordance with a prior art method - a previously prepared mono/di glyceride emulsifier (DIMODAN® CP available from Danisco Ingredients, Denmark) was added. Recipe 2 was in accordance with the present invention. In recipe 2, 1.7% of the fat phase was provided by the above lipase treated fat. The lipase treated fat was added to the fat blend for margarine production and the margarine is produced by standard procedures for margarine production.

Table 8

Recipe	1	2
WATER PHASE		
Water	480	480
Salt	30	30
Skim Milk Powder	30	30
Potassium Sorbate	3	3
EDTA	0.45	0.45
pH	5.5	5.5
FAT PHASE		
Soya 41°	490	481
Soya 35°	490	481
Soya oil	1471	1444
DIMODAN® CP	6.0	-
Lipase	-	51.0
PPM β-carotene	0.5	0.5

Flavourings	0.6	0.6

The fat contacted with lipase generated an emulsifier. The margarine in accordance with the present invention was visually inspected and found to substantially identical in appearance to the conventionally prepared margarine. No separation of the oil and water phases was observed. The margarine in accordance with the present invention was also spread on bread and tasted. The organoleptic properties of the margarine was pleasant and were felt by the taster to be substantially identical to those of the conventionally prepared margarine.

Example 7 -Margarine

In a vessel 1 part of palm oil and 0.15 part of esterase from *Candida* dissolved in sugar (sucrose)/water is added. The reaction is continued for 20 hours at 55°C and then shortly treated by 100°C in order to inactivate the enzyme.

1% of this reaction mixture is added to a fat blend for margarine production and the margarine is produced by standard procedures for margarine production.

The reaction mixture gives good water in oil emulsification properties.

Example 8 -Margarine

In a vessel 1 part of palm oil and 0.15 part of lipase from *Candida* dissolved citric acid/glycerol/water is added. The reaction is continued for 20 hours at 55°C and then shortly treated by 100°C in order to inactivate the enzyme.

1% of this reaction mixture is added to a fat blend for margarine production and the margarine is produced by standard procedures for margarine production.

The reaction mixture gives good water in oil emulsification and also contributes to reduce spattering when the margarine is used for frying.

Example 9 -Margarine

In a vessel 1 part of palm oil and 0.05 part of lipase from *Aspergillus niger* dissolved water is added. The reaction is continued for 20 hours at 40°C and then shortly treated by 100°C in order to inactivate the enzyme.

1% of this reaction mixture is added to a fat blend for margarine production and the margarine is produced by standard procedures for margarine production.

The reaction mixture gives good water in oil emulsification.

Example 10 -Margarine

In a vessel 0.8 part of sun flower oil and 0.2 part of soya protein hydrolysate and 0.05 part of lipase from *Rhizopus arrhizus* is added. The reaction is continued for 2 days at 55°C during vigorous agitation, and then shortly treated by 100°C in order to inactivate the enzyme.

2% of this reaction mixture is added to a fat blend for margarine production and the margarine is produced by standard procedures for margarine production.

The reaction mixture gives good water in oil emulsification.

Example 11 -Ice Cream

In a vessel 0.6 part of palm oil and 0.4 part of milk protein and 0.05 parts of lipase from *Candida* is added. The reaction is continued for 2 days at 55°C and then shortly treated by 100°C in order to inactivate the enzyme.

1% of this reaction mixture is used for ice cream production.

The reaction mixture gives good water in oil emulsification properties and stabilise lipid protein boundaries.

Example 12 - Custard Cream

In a vessel 1 part of palm oil and 0.3 part peptides from soya bean protein and 0.05 parts of lipase from *Candida* is added. The reaction is continued for 3 days at 55°C and then shortly treated by 100°C in order to inactivate the enzyme.

2% of this reaction mixture is used for the production of custard cream.

The reaction mixture gives good water in oil emulsification and contributes to improved stability and mouth feel.

Example 13 - Margarine

In a vessel 0.75 part of soya bean oil, 0.25 part milk protein and 0.05 part of lipase from *Aspergillus niger* is added. The reaction is continued for 3 days at 40°C and then shortly treated by 100°C in order to inactivate the enzyme.

2% of this reaction mixture is added to a fat blend for margarine production and the margarine is produced by standard procedures for margarine production.

The reaction mixture gives good water in oil emulsification.

Example 14 - Sponge Cake

0.05 parts of sugar is dissolved in 0.15 part of glycerol. To this solution 0.75 part of soya bean oil, and 0.05 part of lipase from *Rhizopus arrhizus* is added. The reaction is continued during stirring for 1 day at 45°C and then the reaction mixture, comprising a functional ingredient, is shortly treated at 100°C in order to inactivate the enzyme.

This reaction mixture is used for sponge cake production. The reaction mixture gave good emulsification properties and produced a cake with a stable crumb structure and a good volume.

Sponge Cake Recipe

Ingredients	Gram
Sugar	208
Wheat Flour	188
Corn starch	60
Baking Powder	14
Egg	200
Soya oil	40
Water	110
Functional ingredient	30

Procedure

Mix all ingredients for 6 min. on a Hobart N50 mixer.

Scale 2 x 350 g into round sponge cake tins.

Bake 35 min at 180°C

Example 15 - Soft table margarine

Materials

Palm oil:	Palmotex from Aarhus Oil, Denmark
Glycerol:	Food grade 99.5%
Lipase #1920:	Lipase PS "Amano" from *Pseudomonas cepacia*, available from Amano, Japan
DIMODAN® BP:	Distilled monoglyceride from Danisco Ingredients, Denmark

Palm oil was reacted with at solution of lipase in glycerol according to the following recipe Table 9 and Table 10

Table 9

		1	2	3	4	5
Palmotex, palm oil	g	100	100	100	100	100
Glycerol-lipase solution	g	32	21	11	32	11

Table 10

Glycerol-lipase solution		1	2	3	4	5
Glycerol		90	90	90	95	95
Lipase #1920, solution in water	5100 LUT/g	10	10	10	5	5

Procedure:

2.5 gram of lipase #1920 was dissolved in 15ml of water whilst being stirred at ambient temperature. A glycerol-lipase solution was prepared as disclosed in Table 10. The glycerol lipase solutions were added to the palm oil in accordance with the recipes of Table 9 and incubated at 45 °C for 20 hours.

The samples were then heated to 90 °C for 10 minutes and the upper oil phase was isolated. The isolated oil phases were analysed by GLC. The results obtained are given in Table 11.

Table 11

		1	2	3	4	5
Glycerol	%	2.3	2.1	2	2.2	1.4
Free fatty acid	%	5.7	4.8	4.3	3	2.4
Monoglyceride	%	24.5	24.6	24	25	19.7
Diglyceride	%	47.3	48	47.7	48.2	47.6
Triglyceride	%	20.2	20.5	21.9	21.7	28.8

Table 11 indicates that 20-25% monoglyceride was formed during this enzymatic reaction. These samples were used to prepare a soft table margarine according to the recipe given in Table 12 below

Table 12

	Margarine No.						
	1	2	3	4	5	6	7
Water phase							
Water phase	16	16	16	16	16	16	16
Salt	1	1	1	1	1	1	1
Skim milk powder	1	1	1	1	1	1	1
Potassium sorbate	0.1	0.1	0.1	0.1	0.1	0.1	0.1
EDTA	0.015	0.015	0.015	0.015	0.015	0.015	0.015
pH	5.5	5.5	5.5	5.5	5.5	5.5	5.5
Water phase total	18.1	18.1	18.1	18.1	18.1	18.1	18.1
Fat phase							
Soya, 41 °C	20	20	20	20	20	20	20
Soya, 35 °C	20	20	20	20	20	20	20
Soya oil	60	60	60	60	60	60	60
Fat phase total	81.7	81.02	81.02	81.03	81.08	80.88	81.6
Dimodan BP	0.2						
Sample 1		0.88					
Sample 2			0.88				
Sample 3				0.87			
Sample 4					0.82		
Sample 5						1.02	
Soya lecithin							0.3
β-carotene, ppm	3	3	3	3	3	3	3
Fat phase total	81.9	81.9	81.9	81.9	81.9	81.9	81.9
Flavorings:							
Flavouring 2565*,%	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Flavouring 2712*,%	0.01	0.01	0.01	0.01	0.01	0.01	0.01

* Butter Flavouring 2565 and Butter Flavouring 2712 are available from Danisco Ingredients, Denmark.

Evaluation

Margarine numbers 1 to 7 were evaluated after 4 days storage at 5°C.

Visual :

Margarines 1 to 7 all produced fine and stable margarine.

Margarines 2 to 6 were slightly more firm than 1 and 7.

Organoleptic:

Each of margarines 1 to 7 gave very good melting properties on the tongue.

Microscopy:

Margarines 1, 5, 6 and 7 gave very good dispersions of water particles in the fat phase, with an average diameter of 4-5 μm.

Margarines 2,3 and 4 also produced a fine dispersion with water particles approx. 5μm, but a few water particles of 10 μm were observed.

From the experiment with margarine it can be concluded that the enzymatic inter-esterification of palm oil (Margarine Nos. 1 to 5) can be used to produce margarine which is equal in quality to commercial products. Furthermore, it is shown that these samples can substitute distilled monoglyceride or lecithin for the production of a soft table margarine. The change in firmness observed using interesterified palm oil in place of lecithin can be adjusted by changing the fat composition of the fat phase in the margarine.

Example 16 - Puff pastry margarine

Materials:

Palm oil:	
Palm stearin:	Melting point 55 °C
Palm:	Melting point 43 °C
Rape seed oil:	
Glycerol:	Food grade 99.5%
Lipase #1920:	Lipase PS "Amano" from Pseudomonas *cepacia*
DIMODAN® BP:	Distilled monoglyceride from Danisco Ingredients, Denmark
DIMODAM® PVP:	Distilled monoglyceride from Danisco Ingredients, Denmark
Flavouring O2986:	Butter flavouring available from Danisco Ingredients, Denmark

Palm oil and palm stearin were reacted with at solution of lipase in glycerol according to the following recipe Table 13 and Table 14.

Table 13

		Sample 1	Sample 2
Palmotex, palm oil	g	300	
Palm stearin	g		200
Glycerol-lipase solution 1	g	33	22

Table 14

Glycerol-lipase solution		
Glycerol		90
Lipase #1920, solution in water	5100 LUT/g	10

Procedure:

2.5 g lipase #1920 were dissolved in 15 ml water during stirring at ambient temperature. Glycerol-lipase solution was made as mentioned in Table 14.

The glycerol-lipase solutions were added to the palm oil or palm stearin as shown in Table 13 and incubated at 45°C for 20 hours.

The samples were heated to 90°C for 10 min and the upper oil phase isolated.

Table 15

		Sample 1	Sample 2
Glycerol	%	1.3	2.9
Free fatty acid	%	7.2	6.5
Monoglyceride	%	19.2	16.1
Diglyceride	%	42.3	42.9
Triglyceride	%	29.8	31.5

Table 15 indicate that 15-20 % monoglyceride was formed during this enzymatic reaction.

These samples were used to produce a puff pastry margarine according to the recipe of Table 16.

Table 16

	Margarine			
	1	2	3	4
Water phase				
Water phase	36.9	36.9	36.9	36.9
Salt	2	2	2	2
Sugar	1	1	1	1
Potassium sorbate	0.1	0.1	0.1	0.1
EDTA	0.015	0.015	0.015	0.015
pH	3	3	3	3
Water phase total, %	40	40	40	40
Fat phase				
Palm stearin 55	20	20	20	20
Palm 43	25	25	25	25
Palm oil	45	45	45	45
Rape seed oil	10	10	10	10
Fat total,%	58.8	58.8	54.8	54.8
Dimodan PVP, %	1			
Dimodan BP, %		1		
Sample 1, %			5	
Sample 2, %				5
Lecithin, %	0.2	0.2	0.2	0.2
β-carotene, ppm				
Fat phase total, %	60	60	60	60
Flavouring O2986, %	0.03	0.03	0.03	0.03

Evaluation

Margarines 1 to 4 were evaluated after 2 days storage at 5 °C.

Visual :

Margarines 1 to 4 all produced fine and stable puff pastry margarine

Margarine 1: Soft plastic

Margarine 2: Soft plastic was more soft than Margarine 1

Margarine 3: Soft plastic slightly more soft than Margarine 1

Margarine 4: Soft plastic better than Margarine 1

Microscopy:

Margarine 1 gave very nice dispersions of water particles in the fat phase,

Margarine 2 and Margarine 3 were evaluated equal fine water particles.

Margarine 4 was evaluated slightly better than 1.

Microscopic pictures of the samples is shown in Figures 2 and 3. Margarines 1 to 4 are shown as 1863-1 to 4, respectively.

Conclusion:

From the experiment with puff pastry margarine it can be concluded that the enzymatic interesterification of palm oil or palm stearin can be used to produce margarine which are fully on level with the quality of commercial products.

It has been shown that these samples made by enzymatic interesterification can substitute distilled monoglyceride for the production of a puff pastry margarine.

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in chemistry or related fields are intended to be within the scope of the following claims.

CLAIMS

1. Use of a conversion agent to prepare from a food material a foodstuff comprising at least one functional ingredient, wherein the at least one functional ingredient has been generated from at least one constituent of the food material by the conversion agent.

2. A process for preparing a foodstuff comprising the steps of

(i) providing a food material;

(ii) contacting the food material with a conversion agent such that a functional ingredient is generated by the conversion agent from at least one constituent of the food material.

3. A foodstuff prepared from a food material, wherein the foodstuff comprises at least one functional ingredient, and wherein the at least one functional ingredient has been generated from at least one constituent of the food material by a conversion agent.

4. Invention according to claim 1, 2 or 3 wherein the conversion agent is present in the foodstuff.

5. Invention according to any one of the preceding claims wherein the conversion agent is a catalyst.

6. Invention according to claim 5 wherein the conversion agent is an enzyme.

7. Invention according to claim 6 wherein the enzyme is selected from lipase, esterase, derivatives and mixtures thereof.

8. Invention according to claim 6 or 7 wherein the enzyme is isolated from a plant, an animal or a micro-organism.

9. Invention according to claim 8 wherein the micro-organism is selected from

Aspergillus niger, Rhizopus delemar, Rhizopus arrhizus, Mucor miehei, Pseudomonas sp., Candida rugosa, Pencilium roqueforti, Pencilium cyclopium, Aspergillus tubingensis, Candida cylindracea, Thermomyces lanuginosus, Mucor javanicus, Candida antarctica, Chromobacterium viscosum, Pseudomanas fluorescens, Pseudomonas nitroreducans, Chromobacterium viscosum, Bacillus subtilis mutants and combinations thereof.

10. Invention according to any one of the preceding claims wherein the conversion agent is present in an inactive form or a denatured form in the foodstuff.

11. Invention according to any one of the preceding claims wherein the at least one constituent of the food material is selected from esters, monoglycerides, diglycerides, triglycerides, fats, including lard, tallow and butter fat; fatty acids, fatty acid esters, waxes, wax esters, oils including oils extracted from or derived from palm oil, sunflower oil, soya bean oil, safflower oil, cotton seed oil, ground nut oil, corn oil, olive oil, peanut oil, coconut oil and rape seed oil, proteins, amino acids, protein hydrolysates, peptides (partly hydrolysed protein), a constituent comprising a hydroxy group (-OH), polyvalent alcohols, including glycerol; water, ethanol, sugars including sucrose, fructose, glucose (dextrose), lactose, and galactose; dextrins including maltodextrin, sorbitol, mannitol, fruit acids and hydroxy acids including citric acid, tartaric acid, lactic acid and ascorbic acid; proteins, amino acids, protein hydrolysates, peptides (partly hydrolysed protein); mixtures and derivatives thereof.

12. Invention according to claim 11 wherein the at least one constituent of the food material is selected from monoglycerides, diglycerides, and mixtures thereof.

13. Invention according to any one of the preceding claims wherein the functional ingredient is generated from at least two constituents of the food material.

14. Invention according to any one of the preceding claims wherein the functional ingredient is generated from a first constituent and a second constituent of the food material.

15. Invention according to claim 14 wherein the first constituent and the second constituent are constituents of the foodstuff.

16. Invention according to claim 14 or 15 wherein the first constituent is hydrophobic and/or lipophilic.

17. Invention according to any one of claims 14 to 16 wherein the first constituent is selected from fats, oils, fatty acids, derivatives and mixtures thereof.

18. Invention according to any one of claims 14 to 17 wherein the second constituent is hydrophilic.

19. Invention according to any one of claims 14 to 18 wherein the second constituent is selected from a constituent comprising a hydroxy group (-OH), polyvalent alcohols, including glycerol; water, ethanol, sugars including sucrose, fructose, glucose (dextrose), lactose, and galactose; dextrins including maltodextrin, sorbitol, mannitol, fruit acids and hydroxy acids including citric acid, tartaric acid, lactic acid and ascorbic acid; mixtures and derivatives thereof.

20. Invention according to claims 14 to 18 wherein the second constituent is selected from proteins, amino acids, protein hydrolysates, peptides (partly hydrolysed protein), derivatives and mixtures thereof.

21. Invention according to any one of the preceding claims wherein the foodstuff is selected from baked goods, including breads, cakes, muffins, doughnuts, biscuits, crackers and cookies; confectionery, including candies, caramels, chocolate and puddings; frozen products, preferably frozen dairy products, including ice cream and ice milk; dairy products, including coffee cream, whipped cream, custard cream, milk drinks and yoghurts; meat products, including processed meat products; edible oils and fats, including w/o emulsions, o/w emulsions, margarine shortening and spreads; fine foods, including sauces and mayonnaise.

22. Invention according to claim 1 wherein the foodstuff comprises at least two functional ingredients, and wherein the at least two functional ingredient have been generated from a first constituent of the food material and a second constituent of the food material by a conversion agent.

23. Invention according to claim 22 wherein the first constituent is a constituent comprising at least two ester groups, preferably a triester, more preferably a triglyceride.

24. Invention according to claim 22 or 23 wherein the second constituent is a sugar or a sugar alcohol, more preferably ascorbic acid.

25. Invention according to any one of the preceding claims wherein the at least one functional ingredient comprises or is an emulsifier.

26. Invention according to claim 25 wherein the emulsifier is selected from monoglycerides, diglycerides, and mixtures thereof.

27. Use according to claim 1 substantially as hereinbefore described.

28. Process according to claim 2 substantially as hereinbefore described.

29. Foodstuff according to claim 3 substantially as hereinbefore described.

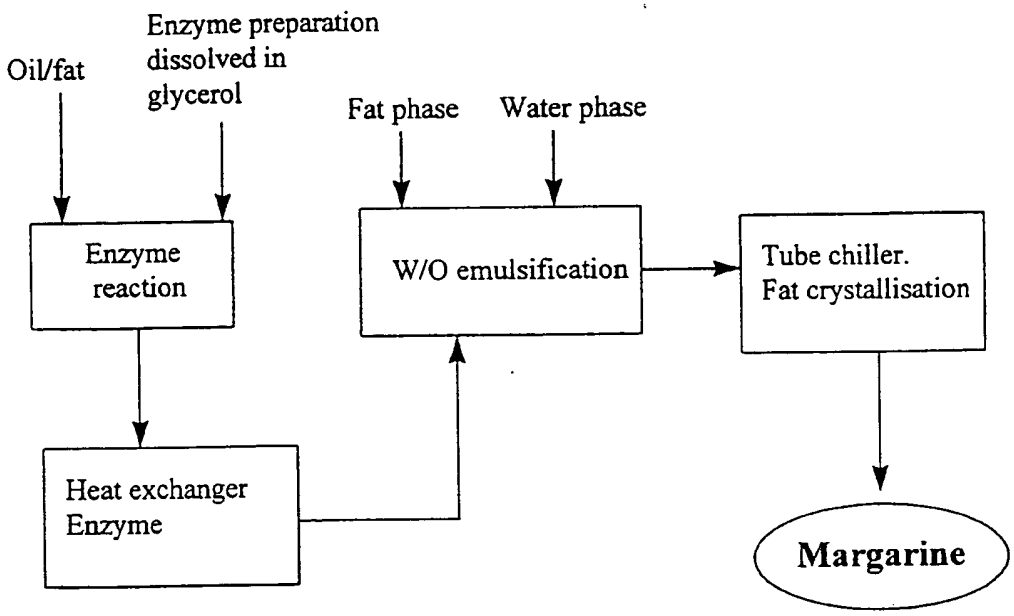

Oil/fat
Enzyme preparation dissolved in glycerol
Fat phase
Water phase
Enzyme reaction
W/O emulsification
Tube chiller. Fat crystallisation
Heat exchanger Enzyme
Margarine

Figure 1

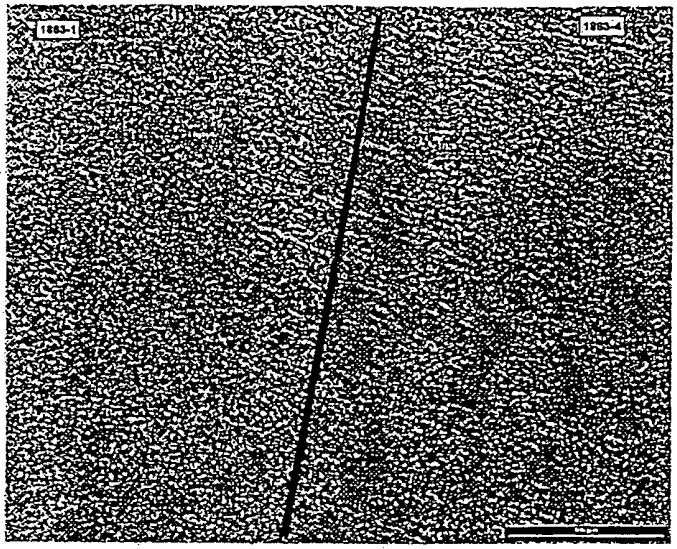


Fig. 2

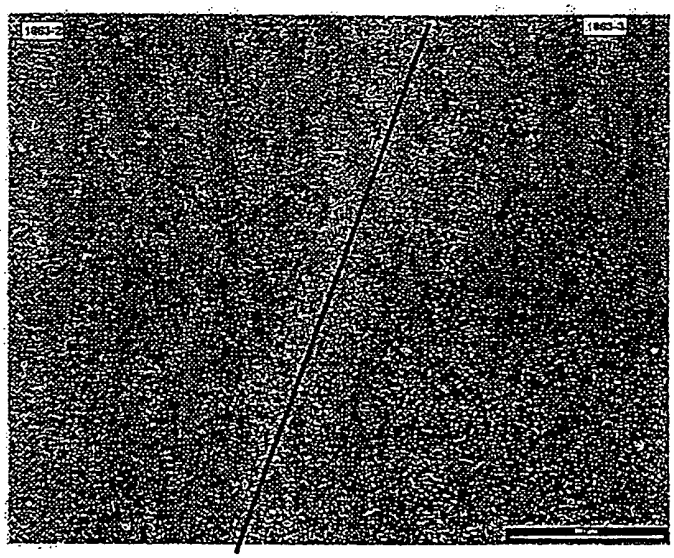


Fig. 3

INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/IB 99/01354

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12P7/64 A23D7/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23D C11C C12P A23L A23G

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	EP 0 882 797 A (UNILEVER) 9 December 1998 (1998-12-09) page 2, line 2 - line 9 page 2, line 41 - line 51 claims 1,2,5	1-29
X	US 5 695 802 A (VAN DEN OUWENHAND) 9 December 1997 (1997-12-09) column 4, line 36 - line 63 examples 3-5,12 claims 15-18	1-29
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-/—

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 November 1999	06. 12. 1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Lepretre, F

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INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/IB 99/01354

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 445 692 A (EUROPEAN ECONOMIC COMMUNITY) 11 September 1991 (1991-09-11) page 2, line 28 - line 30 claim 1	1-29
X	WO 91 06661 A (ENZYTECH, INC.) 16 May 1991 (1991-05-16) page 2, line 13 -page 3, line 19; claims 1,5,11	1-29
X	EP 0 191 217 A (AMANO PHARMACEUTICAL) 20 August 1986 (1986-08-20) page 3, line 18 - line 23 page 6, line 7 - line 20 claim 1	1-29
A	WO 92 14830 A (SCHNEIDER ET AL.) 3 September 1992 (1992-09-03) page 20, line 14 -page 21, line 5 page 2, line 1 - line 18 page 2, line 4 - line 8 page 8, line 12 - line 19 page 24, line 11 - line 20 page 25, line 13 - line 22 example 13 claims 1,44	1-29

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB 99/01354

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-29 relate to an extremely large number of possible products and methods. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the products/methods claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the products and methods wherein the conversion agent is an enzyme (lipase), the first constituent is a fat, and the obtained food product is an emulsifier (see examples, claims 13-24 and pages 7,8,9 and 16 of the application).

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

information on patent family members

Intr ional Application No
PCT/IB 99/01354

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